Toxicology Letters 209 (2012) 173–178

Contents lists available at SciVerse ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Comparative kinetics of organophosphates and oximes with erythrocyte, muscle and brain acetylcholinesterase

Nadja M. Herkert[a], Gregor Freude[b], Ulrich Kunz[b], Horst Thiermann[a], Franz Worek[a,*]

[a] Bundeswehr Institute of Pharmacology and Toxicology, Munich, Germany
[b] Department of Neurosurgery, Military Hospital, Ulm, Germany

ARTICLE INFO

Article history:
Received 3 December 2011
Received in revised form
20 December 2011
Accepted 23 December 2011
Available online 2 January 2012

Keywords:
Acetylcholinesterase
Enzyme kinetics
Erythrocyte
Brain
Oxime
Organophosphorus compound

ABSTRACT

There is an ongoing debate whether oximes can effectively counteract the effects of organophosphorus compounds (OP) on brain acetylcholinesterase (AChE) activity and whether there are differences in the kinetic properties of brain and erythrocyte AChE. In order to investigate the kinetics of AChE from different tissues and species the well established dynamically working in vitro model with real-time determination of membrane-bound AChE activity was adapted for use with brain AChE. The enzyme reactor, that was loaded with brain, erythrocyte or muscle AChE, was continuously perfused with substrate and chromogen while AChE activity was on-line analyzed in a flow-through detector. It was possible to determine the Michaelis–Menten constants of human erythrocyte, muscle and brain AChE which were almost identical. In addition, the inhibition kinetics of sarin and paraoxon as well as the reactivation kinetics of obidoxime and HI 6 were determined with human, swine and guinea pig brain and erythrocyte AChE. It was found that the inhibition and reactivation kinetics of brain and erythrocyte AChE were highly comparable in all tested species. These data support the view that AChE from different tissue has similar kinetic properties and that brain AChE is comparably susceptible toward reactivation by oximes.

1. Introduction

The standard treatment of poisoning by organophosphorus compounds (OP) includes the administration of a muscarinic antagonist, mostly atropine, and an acetylcholinesterase (AChE) reactivator (oxime) (Eyer et al., 2007; Cannard, 2006). Oximes are considered as causal therapy of OP poisoning by removing the phosphyl moiety from the active site serine of AChE, thus restoring the activity of the enzyme (Hobbiger, 1963). The rapid and effective reactivation of inhibited AChE is a prerequisite for the termination of OP-induced cholinergic crisis (Eyer, 2003).

Clinically used, e.g. obidoxime and pralidoxime, as well as most experimental oximes are positively charged pyridinium salts and there is an ongoing debate whether oximes are able to cross the blood–brain-barrier to an extent which enables the reactivation of central nervous AChE (Bodor and Brewster, 1983; Worek et al., 2010). In addition, in vitro studies indicate that there is a significant difference in the reactivation kinetics of oximes between brain and erythrocyte AChE (de Jong and Wolring, 1985; de Jong and Kossen, 1985). Mammalian AChE is encoded by a single gene but subject to post-transcriptional mechanisms and post-translational modifications (Massoulie, 2002). The molecular diversity of AChE located in different tissues is a result of different quaternary associations and various membrane anchors but these differences are not assumed to affect the catalytic unit (Massoulie et al., 1999). Hence, observed kinetic differences between brain and peripheral tissue AChE should not be due to different catalytic properties of the enzyme species but could be a result of sample preparation and differences in the experimental setup.

In fact, kinetic in vitro studies on the inhibition and reactivation of brain AChE are usually undertaken with enzyme solubilized by using detergents in order to eliminate the annoying impact of particulate matter during the spectrophotometric Ellman assay (Rosenfeld et al., 2001; Singh, 1985). However, detergents, e.g. Triton X-100, are known to alter the AChE activity and its kinetic properties (Wille et al., 2011). In addition, there is hardly any study investigating the kinetic properties of membrane-bound brain, erythrocyte and muscle AChE at identical conditions.

Recently, a dynamically working in vitro model for the determination of erythrocyte AChE activity in real-time (Eckert et al.,

Abbreviations: AChE, acetylcholinesterase (E.C. 3.1.1.7); ATCh, acetylthiocholine iodide; AU, absorbance units; DTNB, 5,5′-dithio-bis(2-nitrobenzoic acid); sarin, isopropylmethyl phosphonofluoridate; PXE, paraoxon-ethyl,diethyl-O-4-nitrophenylphosphate; obidoxime, 1,1-(oxybismethylene)bis[4-(hydroxyimino)methyl]pyridiniumdichloride; HI 6, 1-[[[4-(aminocarbonyl)pyridino]methoxy]methyl]-2-[(hydroxyimino)-methyl]pyridinium dichloride monohydrate.

* Corresponding author. Tel.: +49 89 3168 2930; fax: +49 89 3168 2333.
E-mail address: franzworek@bundeswehr.org (F. Worek).

0378-4274/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.toxlet.2011.12.015

2006a,b) was modified for the use of intercostal muscle tissue from different species including man (Herkert et al., 2009; Eckert et al., 2008). With this in vitro model, it was possible to demonstrate that the kinetic properties of erythrocyte and muscle AChE are virtually identical and that easily available erythrocyte AChE may serve as a proper surrogate for muscle AChE.

Now, it was enthralling to investigate whether the dynamic perfusion model could be adapted for use of human and animal brain tissue and to study the kinetic properties of identically treated brain, erythrocyte and muscle AChE.

After having established the dynamic model with brain tissue we intended to determine the Michaelis–Menten kinetics with human brain, erythrocyte and muscle AChE. In addition, we aimed to investigate the inhibition and reactivation kinetics of paraoxon- and sarin-inhibited erythrocyte and brain AChE with HI 6 and obidoxime.

2. Materials and methods

Acetylthiocholine iodide (ATCh) and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were obtained from Sigma (Deisenhofen, Germany) and obidoxime dichloride from Merck (Darmstadt, Germany). HI 6 dichloride monohydrate was provided by Dr. Clement (Defence Research Establishment Suffield, Ralston, Alberta, Canada). Paraoxon-ethyl (paraoxon) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and was freed from disturbing *p*-nitrophenol as described in detail elsewhere (Kiderlen et al., 2005). Sarin (>98% by GC–MS, ^{1}H NMR and ^{31}P NMR) was made available by the Ministry of Defence (Bonn, Germany). Particle filters employed were Millex®-GS, 0.22 μm (Millipore, Eschborn, Germany). All other chemicals were purchased from Merck Eurolab GmbH (Darmstadt, Germany) at the purest grade available.

Sarin stock solutions (0.1%, v/v) were prepared in acetonitrile and paraoxon stock solutions (10 mM) in 2-propanol and were stored at 20 °C and −80 °C, respectively. The solutions were appropriately diluted in distilled water just before the experiment. Oxime stock solutions (200 mM) were prepared in distilled water, stored at −80 °C and diluted as required in 0.1 M phosphate buffer (0.1 M, pH 7.4) on the day of the experiment. All solutions were kept on ice until the experiment.

2.1. Animal blood and brain samples

Heparinized swine whole blood was obtained from the local slaughterhouse in Munich and porcine brain tissue was supplied by the Centre of Preclinical Research, Klinikum rechts der Isar, Technical University Munich.

Male Pirbright-white (Dunkin–Hartley strain) guinea pigs (300–350 g, Charles River, Sulzfeld, Germany) were euthanized with isoflurane and heparinized whole blood was drawn and brain tissue was excised.

All procedures using animals followed animal care regulations and were approved by the local Ethics Committees.

2.2. Human muscle and brain samples

Human intercostal muscle strips (approx. 3 cm × 1 cm × 0.5 cm) were excised from three patients undergoing therapeutic thorax surgery and were kindly supplied by Prof. Dr. R.A. Hatz, Department of Thoracic Surgery, Asklepios Clinic, Gauting, Germany. Muscle samples were stored up to 1 month at −80 °C until preparation of muscle homogenates.

Human brain tissue samples (glioblastoma tissue; approx. 1.0 g) were taken from two patients undergoing therapeutic brain cancer surgery at the Department of Neurosurgery, Military Hospital, Ulm, Germany. All tissue samples were transferred on ice to the Bundeswehr Institute of Pharmacology and Toxicology for further handling. Brain tissue samples were homogenized immediately after arrival at the institute.

Tissue samples from different individuals were not pooled during the further processing.

The study protocol was approved by the local Ethics Committees and the patients gave their written informed consents.

2.3. General experimental procedure

The experiments were performed with the well-described dynamic model (Eckert et al., 2006a, 2008; Herkert et al., 2008, 2010, 2011a). In brief, diluted erythrocytes, muscle or brain homogenates were layered onto a particle filter (Millex®-GS, 0.22 μm, Ø 33 mm) serving as the enzyme reactor which was submerged into a water-bath with the temperature set to 37 °C. To determine control AChE activity, the enzyme reactor was continuously perfused with acetylthiocholine (ATCh; 0.45 mM), DTNB (Ellman's reagent, 0.3 mM) and phosphate buffer (0.1 M, pH 7.4). The total flow rate through the reactor was 0.5 mL/min with the effluent passing a photometer set at 470 nm.

The digitized absorbance values were collected at intervals of 1.6 s. Two HPLC pumps with integrated quaternary low-pressure gradient formers set up the perfusion system that was programmed by a computer using commercial HPLC software.

2.4. Preparation of the enzyme reactor

2.4.1. Erythrocytes

Erythrocytes were prepared from freshly drawn heparinized whole blood as described before (Eckert et al., 2008; Herkert et al., 2010). In brief, red blood cells were washed five times with an approximately threefold volume of 0.1 M phosphate buffer. The resulting sediment was re-suspended in phosphate buffer and adjusted to a final haemoglobin concentration of 5 g/dL. This dilution was stored at −80 °C until preparation use. For each experiment, 80 μL diluted erythrocytes were further diluted to 5 mL with 0.1 M phosphate buffer. Subsequently, 3.2 mL were slowly layered onto the Millex syringe filter unit within 10 min with a peristaltic pump.

The enzyme reactor was inserted at $t = 0$ and perfused with phosphate buffer containing 0.2% gelatin from porcine skin (w/v) for 5 min. A pulse of distilled water followed to facilitate complete haemolysis (5 min) and further flushing with gelatin phosphate buffer ($t = 10$) for 5 min. At $t = 15$ DTNB and acetylthiocholine were added to determine the control enzyme activity ($t = 30$ min).

2.4.2. Muscle tissue

Human muscle tissue homogenate was prepared as described previously (Herkert et al., 2008) and was stored at −80 °C until further use. 150 μL muscle homogenate were further diluted to a total of 5 mL with phosphate buffer and 4.5 mL were slowly layered onto a particle filter within 14 min by the use of a peristaltic pump.

Then, the enzyme reactor was inserted at $t = 0$ and flushed with phosphate buffer for 2 min. To saturate muscle tissue sulfhydryl-groups, DTNB was added for 60 min before acetylthiocholine was added at $t = 60$ to determine the control enzyme activity ($t = 80$).

2.4.3. Brain tissue

Human, swine and guinea pig brain tissue (approx. 1 g) was weighed into a 15 mL glass test-tube, mixed with a threefold volume of phosphate buffer (0.1 M; pH 7.4) and transferred into a 5 mL Potter–Elvehjem homogenizer and further processed, six times for 10 s each at 1100 rpm on ice. The resulting brain homogenate was diluted with additional 17 mL phosphate buffer and stored in aliquots of 700 μL at −80 °C until use. For each experiment 300 μL brain homogenate were diluted to a total of 5 mL with phosphate buffer and 4.5 mL were slowly layered onto a particle filter within 14 min by the use of a peristaltic pump.

The enzyme reactor was inserted at $t = 0$ and flushed with phosphate buffer for 2 min. To saturate brain tissue sulfhydryl-groups, DTNB was added for 60 min before acetylthiocholine was added at $t = 60$ to determine the control enzyme activity ($t = 80$).

2.5. Michaelis–Menten kinetics

For the determination of the Michaelis–Menten kinetics of human erythrocyte, brain and muscle AChE the enzyme reactors were perfused with 8 different acetylthiocholine concentrations ranging from 0.025 to 1 mM. The absorbance was recorded at equilibrium with each concentration. The Michaelis–Menten constant K_m was calculated according to Eq. (1):

$$v = \frac{V_{max} \times [\text{ATCh}]}{K_m + [\text{ATCh}]} \quad (1)$$

2.6. Perfusion protocol for inhibition of acetylcholinesterase and reactivation with oximes

A working solution of sarin (human: 300 nM; swine/guinea pig: 500 nM) or paraoxon (4.4 μM) in distilled water passed the system at 0.05 and 0.1125 mL/min, respectively, resulting in a final concentration of 30 nM (human) or 50 nM sarin (swine/guinea pig) and 1 μM paraoxon.

2.6.1. Erythrocyte AChE

At maximum enzyme activity ($t = 30$), erythrocyte AChE was inhibited with sarin or paraoxon for 60 min, followed by a 10 min washout. For reactivation 44.4 μM obidoxime or 133.2 μM HI 6 were added to the perfusion medium at a flow of 0.1125 mL/min, resulting in end concentrations of 10 μM obidoxime or 30 μM HI 6. At $t = 150$ min the oxime was discontinued and 20 min later the enzyme reactor was replaced by a plain filter without enzyme source to determine the blank value of the complete perfusion medium consisting of phosphate buffer with gelatin, the chromogen DTNB and the substrate acetylthiocholine.

2.6.2. Brain AChE

Starting at maximum enzyme activity ($t = 80$), brain AChE was inhibited with sarin or paraoxon for 60 min, followed by a 10 min washout-phase. For reactivation 44.4 μM obidoxime or 133.2 μM HI 6 were added to the perfusion medium at a flow of 0.1125 mL/min, resulting in end concentrations of 10 μM obidoxime or 30 μM HI 6. At $t = 200$ min the oxime was discontinued and 20 min later the enzyme reactor

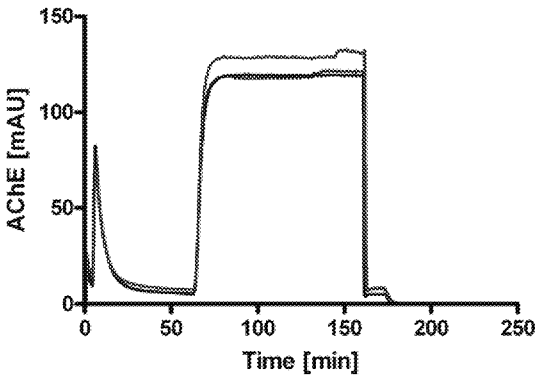

Fig. 1. Time course of human brain AChE activity upon perfusion with DTNB (start at $t = 2$) and ATCh (start at $t = 60$). Original traces of three independent experiments are shown.

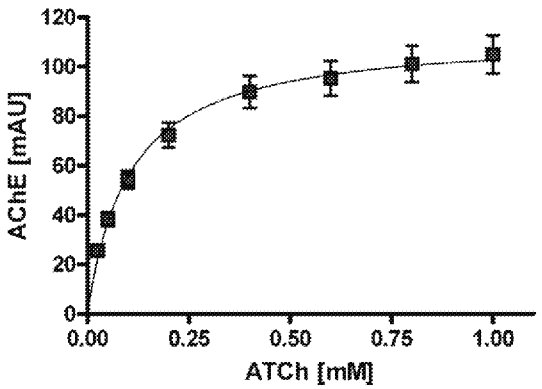

Fig. 2. Calculated Michaelis–Menten curve of human brain AChE upon perfusion with different ATCh concentrations (0.025–1.0 mM). The Michaelis–Menten constants K_m are shown in Table 2.

was replaced by a blank filter without any enzyme source to determine the blank-value of the perfusion medium consisting of phosphate buffer, the chromogen DTNB and the substrate acetylthiocholine.

2.7. Calculations

Processing of experimental data was performed as described before (Eckert et al., 2006a; Herkert et al., 2008). Absorbance values were analyzed by a curve-fitting program (Prism™ Vers. 3.0, GraphPad Software, San Diego, CA, USA).

The time-dependent inhibition of AChE was calculated using Eq. (2):

$$A_t = A_0 \times e^{-kt} \quad (2)$$

The time-dependent oxime-induced reactivation of inhibited AChE was calculated using Eq. (3):

$$A_t = A^0 \times (1 - e^{-kt}) \quad (3)$$

First order kinetics was assumed when the goodness of fit exceeded $R^2 > 0.995$. Data are presented as means ± SD and number of experiments. Statistical differences were analyzed with one-way ANOVA with Bonferroni post hoc comparisons for the Michaelis–Menten kinetics of human erythrocyte, muscle and brain AChE and with the two-tailed Mann–Whitney *U*-test for the inhibition and reactivation kinetics of erythrocyte and brain AChE (GraphPad Prism™ Vers. 3.0). A $p < 0.05$ was considered to be significant.

3. Results

3.1. Development of a brain tissue enzyme reactor

The preparation of brain homogenates in phosphate buffer should enable a direct comparison of brain, erythrocyte and muscle AChE. Pilot experiments showed that careful homogenization with a Potter–Elvehjem homogenizer and constant cooling was essential to preserve brain AChE activity, comparable to AChE activity in muscle tissue. Slow loading of highly diluted brain homogenate on particle filters resulted in an enzyme reactor with reasonable AChE activity. Control experiments verified that the AChE activity did not decrease over time as shown for three original traces (Fig. 1).

3.2. Michaelis–Menten kinetics of human erythrocyte, muscle and brain AChE

Human brain samples were taken from cancer patients and were exclusively glioblastoma tissue. In order to exclude any alteration of the catalytic properties of human brain AChE the Michealis–Menten kinetics of human brain, erythrocyte and muscle AChE were determined. The perfusion of enzyme reactors with different ATCh concentrations enabled the calculation of the Michaelis–Menten constants K_m which were highly comparable (Fig. 2 and Table 1).

Table 1
Michaelis–Menten constants (K_m) of human erythrocyte, muscle and brain AChE.

Enzyme source	K_m [μM]	R^2
Erythrocyte	92.3 ± 3.8	0.997 ± 0.001
Muscle tissue	92.4 ± 11.3	0.998 ± 0.001
Brain tissue	101.1 ± 6.5	0.995 ± 0.001

The data are given as K_m (μM) and are means ± SD of 6 experiments.

3.3. Inhibition of erythrocyte and muscle AChE by sarin and paraoxon

Erythrocyte and brain AChE was inhibited with 30 nM (human) or 50 nM sarin (swine, guinea pig) or 1 μM paraoxon for 60 min, resulting in an inhibited enzyme with a residual activity <0.5% (Fig. 3). The calculation of the pseudo-first order inhibition rate constant k_1 revealed a comparable inhibition kinetic of erythrocyte and brain AChE (Table 2). Nevertheless, a statistical difference of the inhibition rate constants between erythrocyte and brain AChE was found in most cases.

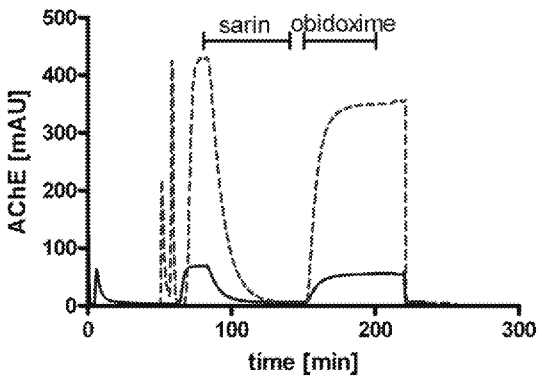

Fig. 3. Time course of AChE activity from Human erythrocytes (hatched red line) and brain tissue (solid blue line) after inhibition with sarin (30 nM) and reactivation with obidoxime (10 μM). Solid blue line: the bioreactor was flushed with 0.3 mM DTNB for 60 min before 0.45 mM ATCh was added. After having reached maximum activity, 30 nM sarin was added for 60 min. After a 10 min washout phase, reactivation was initialized by administration of 10 μM obidoxime. Hatched red line: the enzyme reactor was perfused with phosphate buffer (0.1 M, pH 7.4) containing gelatin, distilled water (to facilitate hemolysis of the erythrocytes) for 5 min before 0.3 mM DTNB and 0.45 mM ATCh were added to determine maximum activity. Inhibition and reactivation followed the same procedure as mentioned above. The *x*-axis of the hatched graph (erythrocytes) were transformed $x + 50$ to align values of the brain experiment for better comparison (for further details see Herkert et al., 2009). Blank values of the perfusion medium consisting of buffer, DTNB and ATCh were collected by removing the bioreactor and replacing it with a filter without any enzyme source. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 2
Rates of inhibition by sarin (k_1) of human, swine and guinea pig AChE (30 nM for human and 50 nM for guinea pig and swine AChE).

AChE	Erythrocyte, k_1 [min^{-1}]	Brain tissue, k_1 [min^{-1}]
Human	0.1087 ± 0.0081	0.1425 ± 0.0169*
Swine	0.1353 ± 0.0105[a]	0.1552 ± 0.0096*
Guinea pig	0.0847 ± 0.0037[a]	0.0807 ± 0.0156

The data are means ± SD of 6 experiments. The goodness of fit of the individual curves was $r^2 > 0.995$.
* $p < 0.05$ between brain and erythrocyte AChE.
[a] From Herkert et al. (2009).

Table 3
Rates of inhibition (k_1) of AChE from human and swine inhibited by paraoxon (1 μM).

AChE	Erythrocyte, k_1 [min^{-1}]	Brain tissue, k_1 [min^{-1}]
Human	0.2950 ± 0.0224	0.2558 ± 0.0565
Swine	0.1266 ± 0.0035[a]	0.1874 ± 0.0077*

The data are means ± SD of 5–6 experiments. The goodness of fit of the individual curves was $r^2 > 0.995$.
* $p < 0.05$ between brain and erythrocyte AChE.
[a] From Herkert et al. (2009).

3.4. Reactivation of sarin- and paraoxon-inhibited AChE by obidoxime and HI 6

Following a 10 min washout phase of the OP, reactivation was started by perfusion with 10 μM obidoxime or 30 μM HI 6. The reactivation of sarin- and paraoxon-inhibited AChE by obidoxime and HI 6 followed first order kinetics (Fig. 3). The determined pseudo-first order reactivation rate constants (k_{obs}) are summarized in Tables 4 and 5. The reactivation velocity was comparable with erythrocyte and brain AChE but again, significant differences were calculated in most cases.

4. Discussion

4.1. Establishment of the brain enzyme reactor

Recently, it could be shown that the dynamic perfusion model, originally developed for use with human erythrocytes, could be successfully adapted for human and animal intercostal muscle homogenate (Herkert et al., 2011a,b; Eckert et al., 2008). In the present study we were able to immobilize human, swine and guinea pig brain tissue and to establish a stable enzyme reactor for the on-line analysis of brain AChE activity. By using highly diluted brain homogenate no clogging of the particle filter occurred and the overall pressure was comparable to particle filters loaded with erythrocytes or muscle homogenate. Control experiments demonstrated that the system was stable for at least 2.5 h and no decrease of brain AChE activity was recorded (Fig. 1).

The preparation of erythrocytes, brain and muscle homogenate in phosphate buffer enabled the investigation of kinetic properties of erythrocyte, brain and muscle AChE at nearly identical conditions. In order to verify the comparability of the kinetic properties of AChE from different tissues the Michaelis–Menten kinetics were determined (Fig. 2). This seemed to be of utmost importance since glioblastoma tissue served as human brain sample and it was not clear whether pathological changes in brain tissue would

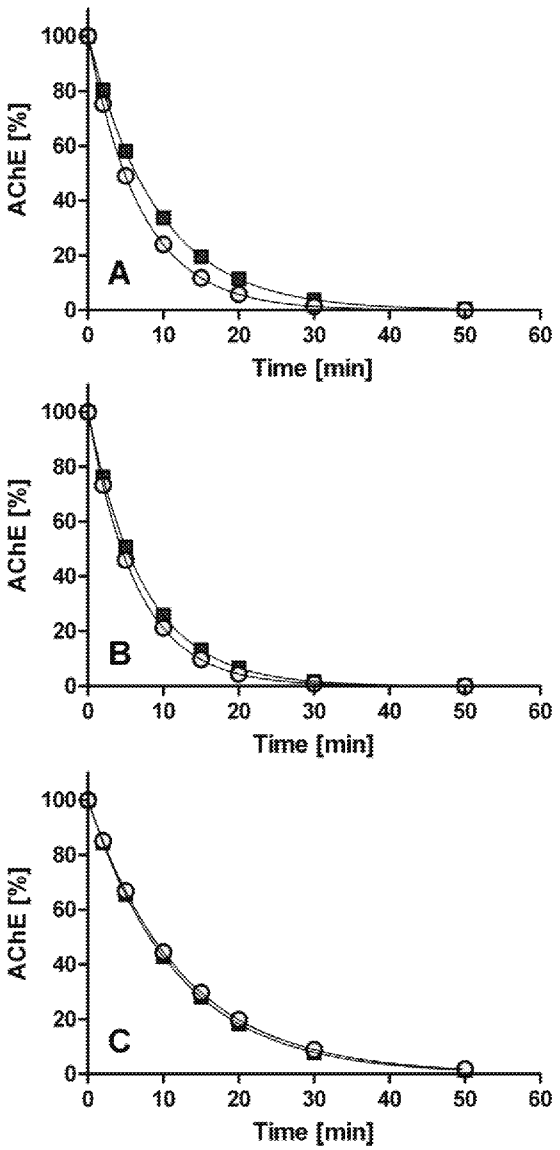

Fig. 4. Calculated time-dependent inhibition of human (A), swine (B) and guinea pig (C) AChE by sarin (30 nM for human, 50 nM for guinea pig and swine AChE). Erythrocyte (red squares) and brain tissue (yellow circles) AChE were calculated using Eq. (2) with the inhibition rate constants given in Table 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 4
Rate constants (k_{obs}) for the reactivation of sarin-inhibited human, swine and guinea pig AChE with oximes.

AChE	Obidoxime (10 μM)		HI 6 (30 μM)	
	Erythrocyte, k_{obs} [min^{-1}]	Brain tissue, k_{obs} [min^{-1}]	Erythrocyte, k_{obs} [min^{-1}]	Brain tissue, k_{obs} [min^{-1}]
Human	0.171 ± 0.009[a]	0.1343 ± 0.0114*	0.211 ± 0.008[a]	0.1576 ± 0.0041*
Swine	0.1374 ± 0.0117[b]	0.1050 ± 0.0109*	0.0419 ± 0.0019[b]	0.0295 ± 0.0048*
Guinea pig	0.1097 ± 0.0016[b]	0.0792 ± 0.0048*	0.0121 ± 0.0003[b]	0.0094 ± 0.0092

The data are means ± S.D. of 5–6 experiments. The goodness of fit of the individual curves was $r^2 > 0.995$.
* $p < 0.05$ between brain and erythrocyte AChE.
[a] From Eckert et al. (2008).
[b] From Herkert et al. (2009).

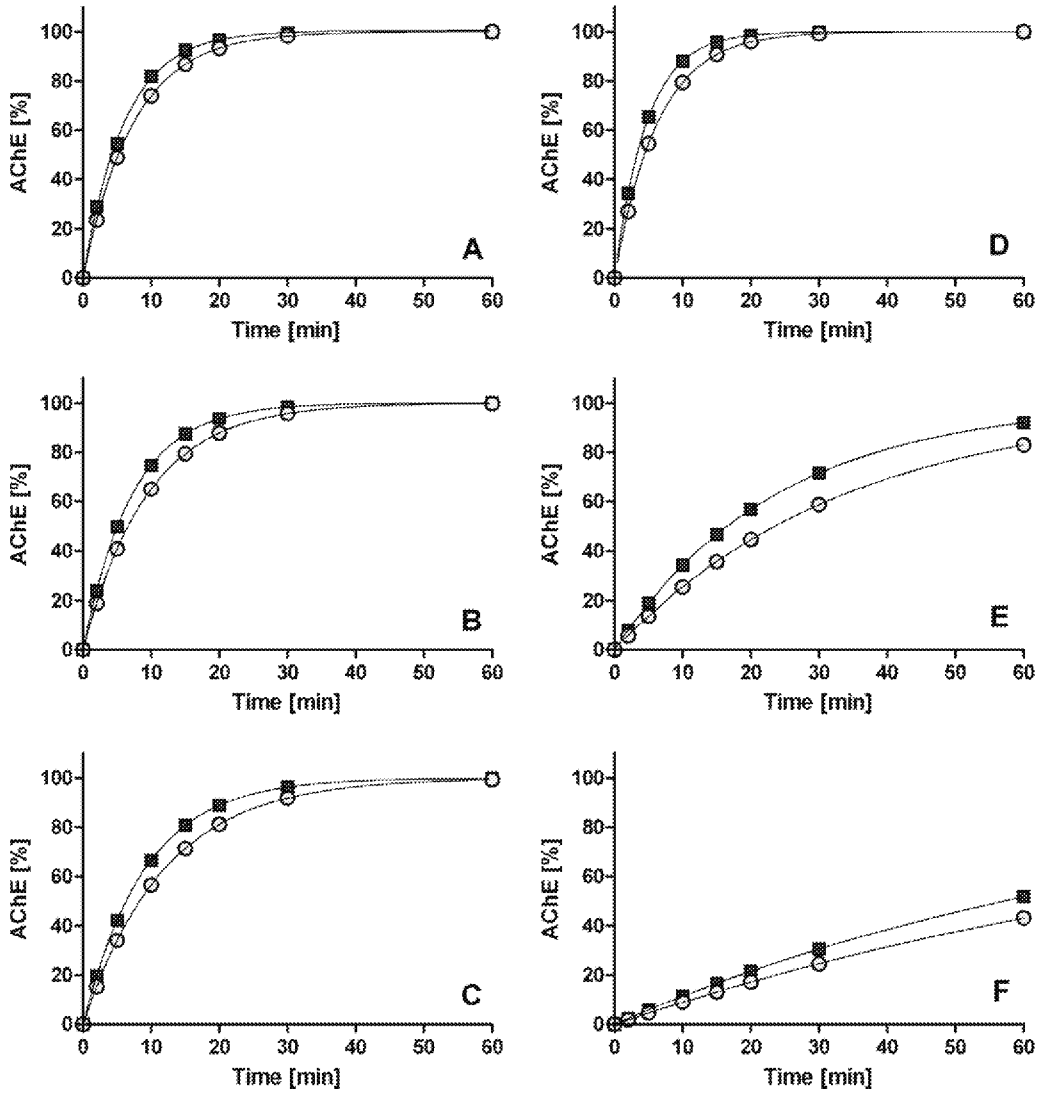

Fig. 5. Calculated time-dependent reactivation of sarin-inhibited human (A), swine (B) and guinea pig (C) AChE (30 nM for human and 50 nM for swine and guinea pig AChE) by obidoxime (10 μM) or by 30 μM HI 6 (human (D), swine (E) and guinea pig (F)). Erythrocyte (red squares) and brain tissue (yellow circles) AChE were calculated using Eq. (3) and the reactivation rate constants given in Table 4 (the data were not corrected for aging of sarin-inhibited AChE). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

also result in an altered enzyme kinetics. Although the maximum activity of brain AChE was substantially lower compared to erythrocyte AChE (Fig. 3) no significant differences of the K_m values were recorded (Table 1). Moreover, the determined K_m of erythrocyte AChE (92.3 μM) was almost identical with the K_m of erythrocyte AChE (95.4 μM) previously determined in a static model (Mast, 1997).

Table 5
Reactivation rate constants (k_{obs}) of paraoxon-inhibited (1 μM) AChE from human and swine with obidoxime (10 μM).

AChE	Obidoxime (10 μM)	
	Erythrocyte, k_{obs} [min^{-1}]	Brain tissue, k_{obs} [min^{-1}]
Human	0.1819 ± 0.0144[a]	0.1207 ± 0.0159*
Swine	0.2238 ± 0.0127[b]	0.1874 ± 0.0077*

The data are means ± SD of 3–6 experiments. The goodness of fit of the individual curves was $r^2 > 0.995$.

* $p < 0.05$ between brain and erythrocyte AChE.

[a] From Eckert et al. (2008).

[b] From Herkert et al. (2009).

4.2. Inhibition and reactivation kinetics

Apart of the ongoing debate whether oximes are able to penetrate the blood-brain-barrier at an adequate rate (Worek et al., 2010) previous studies indicated that kinetic differences between brain and erythrocyte AChE exist (de Jong and Wolring, 1985; de Jong and Kossen, 1985). Hence, the primary aim of the present study was to investigate whether the inhibition and reactivation kinetics of brain and erythrocyte AChE are in fact different if identical experimental conditions are applied. It could be shown that the inhibition of brain and erythrocyte AChE from different species by two model OP, sarin and paraoxon, as well as the reactivation by two oximes was highly comparable. The small differences of the kinetic constants between brain and erythrocyte AChE were found to be significant, which may be attributed to the high reproducibility of the data resulting in remarkable low standard deviations. However, the practical relevance of these differences is minimal. By using the determined constants for inhibition, k_1, and reactivation, k_{obs}, the time dependent inhibition and reactivation was calculated. As shown in Figs. 4 and 5 the decrease and increase of brain and erythrocyte AChE activity was highly comparable.

4.3. Conclusions

The dynamic in vitro model with real-time determination of membrane-bound human AChE activity was successfully adopted for use with human, swine and guinea pig brain AChE. For the first time we could establish an enzyme reactor with immobilized brain AChE and it was possible to investigate the kinetic properties of brain AChE in comparison to erythrocyte and muscle AChE. The determination of the Michaelis–Menten kinetics resulted in almost identical K_m values of the tested enzyme species. In addition, inhibition and reactivation kinetics of brain and erythrocyte AChE were highly comparable. These data support the view that AChE from different tissue has virtually similar kinetic properties and that brain AChE is comparably susceptible toward reactivation by oximes.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

The study was funded by the German Ministry of Defence. The authors are very grateful to A.-K. Preißel (Centre of Preclinical Research, Klinikum rechts der Isar, Technical University Munich, Germany), D. Steinritz and T. Wille for support in obtaining porcine brain and to R.A. Hatz and S. Schulz (Department of Thoracic Surgery, Asklepios Clinic, Gauting, Germany) for donating human muscle samples. In addition, the authors want to thank L. Windisch, T. Hannig and S. Muschik for processing the whole-blood samples and for preparing the guinea pig brain and J. Letzelter for her skilful and engaged technical assistance.

References

Bodor, N., Brewster, M.E., 1983. Problems of delivery of drugs to the brain. Pharmacol. Ther. 19, 337–386.

Cannard, K., 2006. The acute treatment of nerve agent exposure. J. Neurol. Sci. 249, 86–94.

de Jong, L.P.A., Kossen, S.P., 1985. Stereospecific reactivation of human brain and erythrocyte acetylcholinesterase inhibited by 1,2,2-trimethylpropyl methylphosphonofluoridate (soman). Biochim. Biophys. Acta 830, 345–348.

de Jong, L.P.A., Wolring, G.Z., 1985. Aging and stereospecific reactivation of mouse erythrocyte and brain acetylcholinesterases inhibited by soman. Biochem. Pharmacol. 34, 142–145.

Eckert, S., Eyer, P., Herkert, N., Bumm, R., Weber, G., Thiermann, H., Worek, F., 2008. Comparison of the oxime-induced reactivation of erythrocyte and muscle acetylcholinesterase following inhibition by sarin and paraoxon, using a perfusion model for the real-time determination of membrane-bound acetylcholinesterase activity. Biochem. Pharmacol. 75, 698–703.

Eckert, S., Eyer, P., Mückter, H., Worek, F., 2006a. Development of a dynamic model for real-time determination of membrane-bound acetylcholinesterase activity upon perfusion with inhibitors and reactivators. Biochem. Pharmacol. 72, 358–365.

Eckert, S., Eyer, P., Mückter, H., Worek, F., 2006b. Kinetic analysis of the protection afforded by reversible inhibitors against irreversible inhibition of acetylcholinesterase by highly toxic organophosphorus compounds. Biochem. Pharmacol. 72, 344–357.

Eyer, P., 2003. The role of oximes in the management of organophosphorus pesticide poisoning. Toxicol. Rev. 22, 165–190.

Eyer, P., Szinicz, L., Thiermann, H., Worek, F., Zilker, T., 2007. Testing of antidotes for organophosphorus compounds: experimental procedures and clinical reality. Toxicology 233, 108–119.

Herkert, N., Eckert, S., Eyer, P., Bumm, R., Weber, G., Thiermann, H., Worek, F., 2008. Identical kinetics of human erythrocyte and muscle acetylcholinesterase with respect to carbamate pre-treatment, residual activity upon soman challenge and spontaneous reactivation after withdrawal of the inhibitors. Toxicology 246, 188–192.

Herkert, N.M., Aurbek, N., Eyer, P., Thiermann, H., Worek, F., 2010. Comparative study of oxime-induced reactivation of erythrocyte and muscle AChE from different animal species following inhibition by sarin or paraoxon. Toxicol. Lett. 194, 94–101.

Herkert, N.M., Lallement, G., Clarencon, D., Thiermann, H., Worek, F., 2009. Comparison of the oxime-induced reactivation of rhesus monkey, swine and guinea pig erythrocyte acetylcholinesterase following inhibition by sarin or paraoxon, using a perfusion model for the real-time determination of membrane-bound acetylcholinesterase activity. Toxicology 258, 79–83.

Herkert, N.M., Schulz, S., Wille, T., Thiermann, H., Hatz, R.A., Worek, F., 2011a. Pre- and post-treatment effect of physostigmine on soman-inhibited human erythrocyte and muscle acetylcholinesterase in vitro. Toxicol. Appl. Pharmacol. 253, 7–13.

Herkert, N.M., Thiermann, H., Worek, F., 2011b. In vitro kinetic interactions of pyridostigmine, physostigmine and soman with erythrocyte and muscle acetylcholinesterase from different species. Toxicol. Lett. 206, 41–46.

Hobbiger, F., 1963. Reactivation of phosphorylated acetylcholinesterase. In: Koelle, G.B. (Ed.), Cholinesterases and Anticholinesterase Agents. Springer-Verlag, Berlin, pp. 921–988.

Kiderlen, D., Eyer, P., Worek, F., 2005. Formation and disposition of diethylphosphoryl-obidoxime, a potent anticholinesterase that is hydrolysed by human paraoxonase (PON1). Biochem. Pharmacol. 69, 1853–1867.

Massoulie, J., 2002. The origin of the molecular diversity and functional anchoring of cholinesterases. Neurosignals 11, 130–143.

Massoulie, J., Anselmet, A., Bon, S., Krejci, E., Legacy, C., Morel, N., Simon, S., 1999. The polymorphism of acetylcholinesterase: post-translational processing, quaternary associations and localization. Chem. Biol. Interact. 119–120, 29–42.

Mast, U., 1997. Reaktivierung der Erythrozyten-Acetylcholinesterase durch Oxime. Thesis, München.

Rosenfeld, C., Kousba, A., Sultatos, L.G., 2001. Interactions of rat brain acetylcholinesterase with the detergent Triton X-100 and the organophosphate paraoxon. Toxicol. Sci. 63 (2), 208–213.

Singh, A.K., 1985. Kinetic analysis of inhibition of brain and red blood cell acetylcholinesterase and plasma cholinesterase by acephate or methamidophos. Toxicol. Appl. Pharmacol. 81, 302–309.

Wille, T., Thiermann, H., Worek, F., 2011. Effect of different buffers on kinetic properties of human acetylcholinesterase and the interaction with organophosphates and oximes. Arch. Toxicol. 85, 193–198.

Worek, F., Aurbek, N., Thiermann, H., 2010. CNS effects of oximes in nerve agent poisoning—fact or fiction? In: Weissman, B.A., Raveh, L. (Eds.), The Neurochemical Consequences of Organophosphate Poisoning in the CNS. Transworld Research Network, Kerala, pp. 93–110.